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Evaluation of the Automated Haematology Analyser Sysmex NE-8000

By *Katrien Devreese, E. De Logi, Christiane Francart, B. Heyndrickx, J. Philippé* and *G. Leroux-Roels*

Centraal Laboratorium, Universitair Ziekenhuis Gent, Gent, Belgium

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Summary: The automated haematology analyser Sysmex NE-8000 was compared with the Technicon H-1, the automated analyser routinely used in our laboratory, and with manual cell differentiation results.

One hundred and seventy samples from the daily routine workload, comprising specimens from healthy adults and patients with various ailments, were analysed on the Sysmex NE-8000 and the Technicon H-1. A manual-400 leukocyte differential count was performed on each specimen. Comparison of the results from the two blood cell counters showed good correlation ($r > 0.9$) for the white blood cell count, haemoglobin, haematocrit and platelet count. For the red blood cell count and mean cellular volume, the correlation coefficients were greater than 0.8. In the leukocyte differential count, Sysmex NE-8000 and Technicon H-1 showed good correlations for the neutrophil ($r = 0.953$), lymphocyte ($r = 0.763$), and eosinophil counts ($r = 0.904$). Correlation coefficients were very low for monocyte ($r = 0.130$) and basophil counts ($r = 0.006$). Correlation between the manual-400 method and the electronic leukocyte differential count showed similar results.

Two hundred and twenty six normal and abnormal samples were compared with respect to morphology flagging with the two analysers, using the manual differentiation as the reference method. The abnormal specimens were representative of the range of leukocyte abnormalities seen in our laboratory. Sensitivity for detecting blasts was equal for both analysers. Sysmex NE-8000 was much more sensitive for detecting immature granulocytes than Technicon H-1. Low ranges of atypical lymphocytes were missed by Sysmex NE-8000. Left shift was also frequently missed.

During the evaluation period, Sysmex NE-8000 was very easy to handle and no instrument malfunctions were met. The Sysmex NE-8000 is well suited for routine blood cell analysis and is a valuable tool for the diagnosis and screening of various haematological abnormalities.

Introduction

In the past decades the technology used in the haematology routine laboratory has vastly changed. First, traditional procedures for cell enumeration have been replaced by electronic counting methods. More recently, techniques have been developed that are also displacing the time consuming manual microscopic examination of blood smears. The newest instruments perform accurate cell counts for leukocytes, erythrocytes, and platelets, and provide a five-part differential count reported as neutrophils, eosinophils, basophils, lymphocytes, and monocytes. The automated leukocyte differential count has attracted particular attention (1–4). The Sysmex NE-8000 has recently been introduced into the market. This automated haematology analyser with high throughput uses the technology of radio frequency and direct current measurement for cell counting and differentiation (5, 6).

In the present study we have compared the results of the Sysmex NE-8000 with those of the Technicon H-1, the instrument routinely used in our laboratory, which uses the principle of light scattering and cytochemical staining for cell counting and differentiation (7, 8). The leukocyte differential count of the Sysmex NE-8000 is compared with the differential count of the

Technicon H-1, and with manually performed white blood cell differential counts, which were considered as the 'gold standard' throughout this study.

Materials and Methods

Materials, samples

Evaluation was performed in the Central Laboratory of the Universitair Ziekenhuis of Gent, where the daily routine workload for cell counting and differentiation comprises about two hundred blood samples. Samples from in- and outpatients were analysed without selection. During the evaluation period 2038 blood samples were analysed, 143 (7.0%) of which showed abnormalities. Two hundred and twenty six blood samples were chosen at random. These included samples from healthy adults and from patients with various pathologies.

All samples were collected by venipuncture in vacutainer tubes containing EDTA-K_3 (Venoject, Terumo Corp., Tokyo, Japan). Blood samples were kept at room temperature and analysed on a Sysmex NE-8000 and a Technicon H-1, within 4 hours after collection.

Manual method

To reduce the subjective and qualitative aspects of the classical single-slide, 100-cell differential count (manual-100), we employed the modified procedure of the U. S. National Committee for Clinical Laboratory Standards (NCCLS), the H20-T (9).

Normal and abnormal blood specimens were examined. The pathological specimens encountered during the evaluation period were representative of the range of leukocyte abnormalities seen in our laboratory throughout the year. From each blood specimen two blood films were prepared with an ADC-500 spinner (Abbott, Diagnostics Division, Ottignies, Belgium). The blood films were air-dried and stained mechanically with *Wright*'s stain in a Hema-Tek (Ames Company, Division Miles Lab. Inc., Elkhart, Indiana U. S. A.). The stained slides were independently examined by four examiners who cumulatively reported a 400-cell leukocyte differential count (manual-400). A light microscope (Laborlux 11, Wild Leitz, Wetzlar, Germany) (magnification 10 × 50) was used. Manual-400 results were obtained by calculating the average of the four individual manual-100 results for each parameter: neutrophils (segmented and stabcells), lymphocytes, monocytes, eosinophils, and basophils. Each examiner also reported the morphological abnormalities seen in the leukocyte or erythrocyte population. A morphology flag was assigned to a specific specimen when the flag appeared in two of the four manual examinations (10).

Apparatus

The Sysmex NE-8000 haematology analyser (Toa Medical Electronics Corp., Kobe, Japan), an automated 23-parameter (tab. 1) blood cell counter, was compared with the Technicon H-1 (Technicon Instruments Corp. Tarrytown, New York).

Evaluation methods

Comparison of the two blood cell counters

One hundred and seventy patient specimens containing normal, high and low analyte levels were examined on both the Sysmex NE-8000 and the Technicon H-1. Correlation of results for WBC, RBC, HGB, HCT, PLT, MCV, NEUT #, LYMPH #, MONO #, EO #, and BASO # on both instruments was calculated.

Tab. 1. Parameters determined by the Sysmex NE-8000

WBC:	white blood cells
RBC:	red blood cells
HGB:	haemoglobin
HCT:	haematocrit
MCV:	mean corpuscular volume
MCH:	mean corpuscular haemoglobin
MCHC:	mean corpuscular haemoglobin concentration
PLT:	platelet count
REW-SD:	red blood cell distribution width standard deviation
RDW-CV:	red blood cell distribution width coefficient of variation
MPV:	mean platelet volume
PDW:	platelet distribution width
P-LCR:	platelet large cell ratio
NEUT %:	neutrophil percent
LYMPH %:	lymphocyte percent
MONO %:	monocyte percent
EO %:	eosinophil percent
BASO %:	basophil percent
NEUT #:	neutrophil count
LYMPH #:	lymphocyte count
MONO #:	monocyte count
EO #:	eosinophil count
BASO #:	basophil count

Leukocyte differential count

To study the correlation between the manual and both electronic leukocyte differential counts, 170 samples were assayed.

The following abnormalities were observed: blast cells, immature granulocytes, atypical lymphocytes, left shift, increased or decreased number of subpopulations of leukocytes, abnormal erythrocyte-morphology or -colour. Normal or abnormal blood specimens were analysed each three times consecutively on the Sysmex NE-8000 and the Technicon H-1. All analyses were performed within the suggested time limit (4 hours after venipuncture) (9).

A manual 400-cell leukocyte differential count was performed for each specimen. All leukocyte subpopulations were reported in relative percent (as in the manual-100 differential count), then the absolute counts were calculated and referred to the mean of the total leukocyte count of the Sysmex NE-8000 and the Technicon H-1. Correlation coefficients for electronic vs. manual-400 differential count were calculated for NEUT #, LYMPH #, MONO #, EO #, and BASO #.

Linear regressions for Sysmex NE-8000 vs. manual-400 count were plotted for NEUT #, LYMPH #, MONO #; EO #.

Morphology flagging

To evaluate the morphology flags, which are indicators of possible abnormality, 226 specimens were analysed. Morphology flags were assigned when the flag appeared in two of the three electronic counts or in two of the four manual counts (9). The following morphology flags were considered for each specimen: blasts, immature granulocytes, atypical lymphocytes, erythrocyte morphology (aniso-, poikilo-, micro-, macro-, schisto- and echinocytosis, target cells), erythrocyte colour (hypo-, hyperchromia, and polychromasia), and left shift.

Mark limits for Sysmex NE-8000 and Technicon H-1 were set for mean corpuscular volume at 80—99 fl, for mean corpuscular haemoglobin concentration at 5.11—5.73 mmol/l and for red cell distribution width at 11—14.5%. The samples examined during this survey happened to contain only low numbers of

atypical lymphocytes (< 2%). In the manual-400 count, a left shift was assigned when there were more then 8% neutrophil stabcells.

For each flagging, samples were classified as positive or negative. With respect to flagging results, comparisons were made between Sysmex NE-8000 and the manual-400 count, between Sysmex NE-8000 and Technicon H-1, and between Technicon H-1 and the manual-400 count. Ratios for true and false positive and negative samples were calculated.

Data analysis

The relation between the results obtained with both analysers and the manual-400 method was calculated by linear regression analysis according to *Deming* (11). The *Pearson* rank correlation coefficient was calculated for the corresponding parameters.

Results

Comparison of the two blood cell counters

Results of the comparison of both analysers for WBC, RBC, HGB, HCT, PLT, MCV, NEUT #, LYMPH #, MONO #, EO #, and BASO # are shown in table 2. The slopes of the regression lines were close to 1.0 for leukocytes, erythrocytes, haemoglobin, haematocrit, thrombocytes, mean corpuscular volume, neutrophils and lymphocytes. The correlation coefficient (r) was 0.006 for the basophil count and 0.103 for the monocyte count. A negative slope of the regression line was observed for the basophil count.

Leukocyte differential count

Comparison of the manual-400 and the Sysmex NE-8000 leukocyte differential count is shown in table 3. The linear regression curves for NEUT #, LYMPH #, MONO #, and EO # are depicted in figure 1a–d. The slopes of the regression curves were close to 1.0 for the neutrophil and lymphocyte count. The slope of the regression curve of the basophil count was negative. For the monocyte and basophil counts the correlation coefficients were 0.261 and 0.001 respectively. Table 4 compares the manual-400 and the Technicon H-1 leukocyte differential count. The results are comparable with those found for the correlation between the manual-400 and the Sysmex NE-8000 leukocyte differential count. However, in the

Tab. 2. Comparison of Sysmex NE-8000 and Technicon H-1

Parameter		Correlation coefficient r	Regression analysis y = ax + b*		
			a	b	Sxy**
Leukocytes	(10^9/l)	0.997	1.006	0.191	0.599
Erythrocytes	(10^{12}/l)	0.865	0.963	0.296	0.330
Haemoglobin	(mmol/l)	0.985			
Haematocrit	(l/l)	0.931	1.049	−0.052	2.092
Thrombocytes	(10^9/l)	0.974	1.111	8.204	25.744
Mean corpuscular volume	(fl)	0.882	0.911	8.795	2.232
Neutrophils	(10^9/l)	0.953	1.045	0.028	0.851
Lymphocytes	(10^9/l)	0.763	0.942	0.105	0.446
Monocytes	(10^9/l)	0.103	0.234	0.359	0.297
Eosinophils	(10^9/l)	0.903	0.893	0.021	0.046
Basophils	(10^9/l)	0.006	−0.876	0.142	0.506

* Linear regression of y(Sysmex NE-8000) = a x(Technicon H-1) + b
** Standard error of y estimated
170 samples were analysed

Tab. 3. Comparison of manual leukocyte differential count (manual-400) and Sysmex NE-8000 leukocyte differential count

Parameter		Correlation coefficient r	Regression analysis y = ax + b*		
			a	b	Sxy**
Neutrophils	(10^9/l)	0.963	1.015	0.225	0.754
Lymphocytes	(10^9/l)	0.893	0.827	0.200	0.300
Monocytes	(10^9/l)	0.261	0.402	0.242	0.269
Eosinophils	(10^9/l)	0.693	0.704	0.047	0.082
Basophils	(10^9/l)	0.001	−0.450	0.105	0.508

* Linear regression of y(Sysmex NE-8000) = a x(manual-400) + b
** Standard error of y estimated
170 samples were analysed

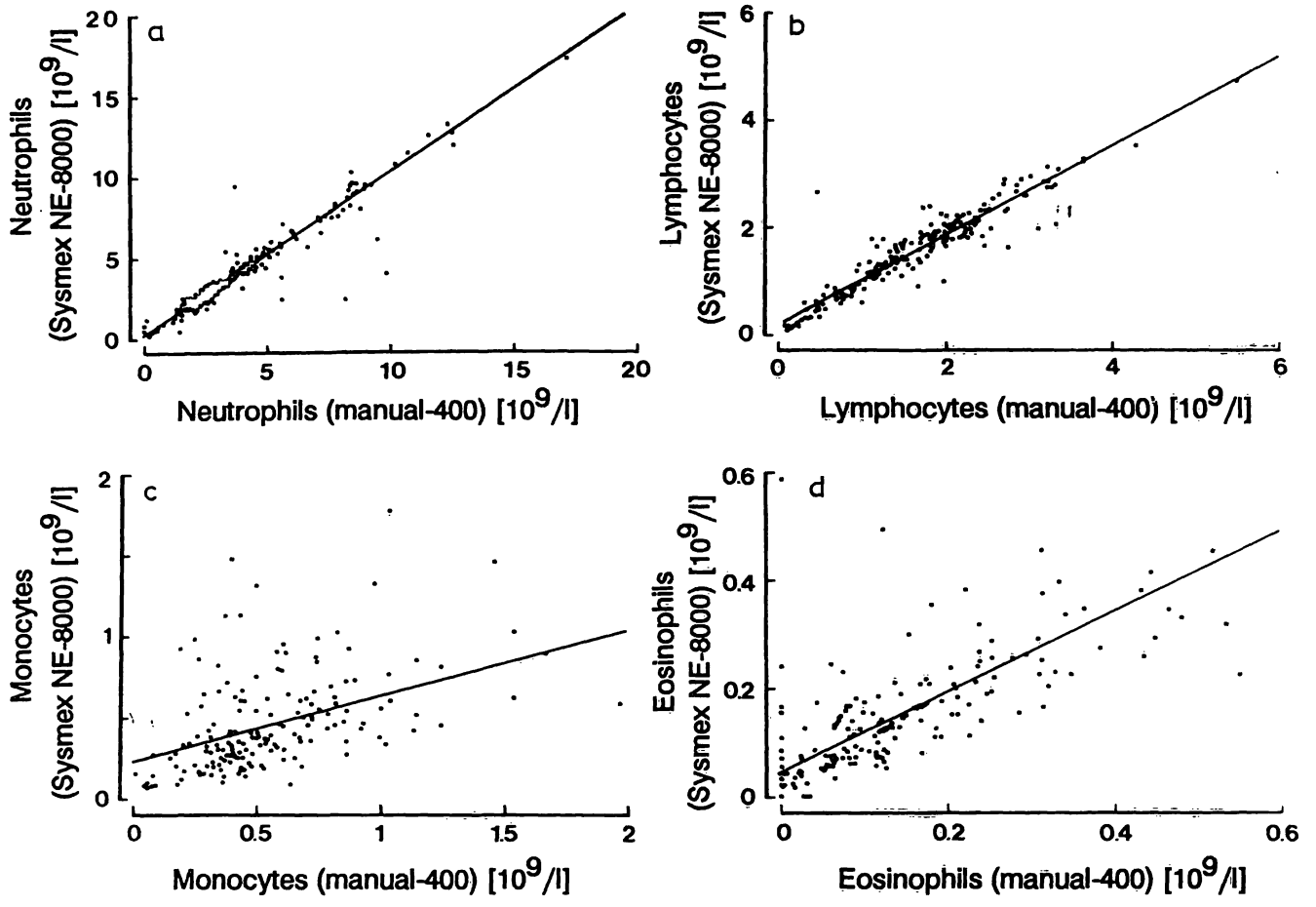

Fig. 1a–d. Graphs of correlation between Sysmex NE-8000 and manual-400 (N = 170) for NEUT # (a), LYMPH # (b), MONO # (c), and EO # (d).
For abbreviations see table 1.
For equations of linear regression see table 3.

Tab. 4. Comparison of manual leukocyte differential count (manual-400) and Technicon H-1 leukocyte differential count

Parameter		Correlation coefficient r	Regression analysis y = ax + b*		
			a	b	Sxy**
Neutrophils	(10^9/l)	0.975	0.954	0.266	0.583
Lymphocytes	(10^9/l)	0.787	0.720	0.371	0.392
Monocytes	(10^9/l)	0.158	0.427	0.240	0.392
Eosinophils	(10^9/l)	0.734	0.811	0.031	0.081
Basophils	(10^9/l)	0.128	0.482	0.043	0.042

* Linear regression of y(Technicon H-1) = a x(manual-400) + b
** Standard error of y estimated
170 samples were analysed

latter, the slope of the regression curve of the basophil count was positive.

Morphology flagging

Table 5 shows the classification of each sample as positive or negative for the morphology flagging. With respect to alarm flagging, Sysmex NE-8000 is compared with the manual-400 method (tab. 5a), Technicon H-1 with the manual-400 method (tab. 5b), and Sysmex NE-8000 with Technicon H-1 (tab. 5c). Using the manual-400 method as the reference method, the ratio of true positives reported by the Sysmex NE-8000 to all manual positives for detecting blasts, was 0.69. There were four false negative results. However, in two of the four cases the abnormality would have been detected anyway; one of the four false negative samples was flagged as a basophilia of 73% and the second sample had an incomplete leukocyte differential count. After correction for these

Tab. 5a–c. Ratios of true and false positive and negative samples* (N = 226) for morphology flagging

Morphology flag	True positives	True negatives
	true positives + false negatives	true negatives + false positives
a) Sysmex NE-8000 (screening method) versus manual-400 (reference method)		
Blasts	9/13 (0.69)**	202/213 (0.95)
Immature granulocytes	31/44 (0.70)***	160/182 (0.88)
Atypical lymphocytes	0/51 (0)	171/174 (0.98)
Left shift	7/24 (0.29)	155/176 (0.88)
Erythrocyte morphology	35/89 (0.39)	135/137 (0.99)
Erythrocyte colour	10/81 (0.12)	144/145 (0.99)
b) Technicon H-1 (screening method) versus manual-400 (reference method)		
Blasts	11/13 (0.85)	211/213 (0.99)
Immature granulocytes	8/44 (0.18)****	178/182 (0.98)
Atypical lymphocytes	21/51 (0.41)	150/174 (0.86)
Left shift	12/25 (0.48)	181/201 (0.90)
Erythrocyte morphology	68/89 (0.76)	116/137 (0.85)
Erythrocyte colour	52/81 (0.64)	122/145 (0.84)
c) Sysmex NE-8000 (screening method) versus Technicon H-1 (reference method)		
Blasts	8/12 (0.67)	202/214 (0.94)
Immature granulocytes	5/13 (0.38)	164/213 (0.77)
Atypical lymphocytes	1/45 (0.02)	178/180 (0.99)
Left shift	5/28 (0.18)	149/173 (0.86)
Erythrocyte morphology	37/89 (0.42)	137/137 (1.00)
Erythrocyte colour	11/75 (0.15)	151/151 (1.00)

* True positive = sample flagged by reference method and screening method
True negative = sample not flagged by reference method nor by screening method
False negative = sample flagged by reference method but not by screening method
False positive = sample flagged by screening method but not by reference method
** 0.85 after correction for other abnormalities
*** 0.82 after the same correction
**** 0.22 after correction

two cases, there were only two false negative results, resulting therefore in a true positive ratio of 0.85 instead of 0.69. The same ratio (0.85) for detecting blasts was obtained with the Technicon H-1. In the detection of immature granulocytes, the ratio of the true positives reported by the Sysmex NE-8000 to all manual positives, can also be corrected by taking into consideration flaggings other than the one for "immature granulocytes". Five of the thirteen false negative results for immature granulocytes were flagged by 'incomplete leukocyte differentiation'. The ratio for detecting immature granulocytes thus became 0.82. The ratio for Technicon H-1, referred to the manual-400 method as a reference test, remained at only 0.22, even after correction for eight of the thirty six false negative results (tab. 5b). These eight false negative results were due to the large amount of large unstained cells present. The low numbers of atypical lymphocytes, in the examined samples, were not detected by Sysmex NE-8000. In the same samples, Technicon H-1 detected atypical lymphocytes with a sensitivity of 0.41. Using the manual-400 method as a reference, both blood cell counters frequently gave false negative results for left shift of the granulocytes. Sysmex NE-8000 produced 17 (71%) false negative results in 24 manual positives, and Technicon H-1 produced 13 (52%) false negative results in 25 manual positives. The red blood cell morphology abnormalities were often not detected by Sysmex NE-8000. Fifty four (61%) false negative results were given by Sysmex NE-8000, whereas Technicon H-1 gave 21 (24%) false negative results in 89 manual-positives. Sysmex NE-8000 gave even more false negative results for the colour abnormalities of the red blood cells. The true positive ratio was 0.12 for Sysmex NE-8000 and 0.64 for Technicon H-1. The sensitivity of erythrocyte morphology flaggings by Sysmex NE-8000 and Technicon H-1 was 0.39 and 0.76 respectively.

Discussion

We compared the results from the newly introduced haematology analyser, Sysmex NE-8000, with those from the Technicon Technicon H-1, the instrument routinely used in our laboratory. The electronic leukocyte differential counts were also compared with the manual-400 leukocyte differentiation.

The numerical results of the Sysmex NE-8000 and the Technicon H-1 correlated very well for leukocytes, erythrocytes, haemoglobin, haematocrit, platelet count, mean corpuscular volume, neutrophil, lymphocyte, and eosinophil count. However, for the monocyte and basophil counts, the correlation was very weak or absent. This may be the result of the different cell identification method used by both analysers. Similar results were found for correlations between the Sysmex NE-8000 and the Technicon H-6000 (12). This lack of correlation for monocyte and basophil counts not only existed for the two automated analysers. But also for each of the analysers and the manual-400 leukocyte differential count. For the monocyte count the four examiners obtained a significantly higher level of agreement with each other than with the automated analysers. Similar observations have been made by other investigators who compared manual leukocyte differential counts with those obtained with automated analysers (13).

The sensitivity for leukocyte abnormalities found in the present study corresponds with the manufacturer's specifications for leukocyte flagging. The instrument's sensitivity for detecting blasts is 50% for the samples containing one blast per 100 white blood cells, and 100% for the samples containing 2% or more blasts. The instrument's sensitivity for detecting immature granulocytes is 100% for samples containing 3% or more immature granulocytes, and approximately 60% when the sample contains 1 or 2% immature granulocytes. The instrument detects atypical lymphocytes in samples containing at least 2 or 3% atypical lymphocytes (14). In addition to the suspect messages, use of other abnormal and flagging criteria, including variation from normal numbers of leukocyte counts and differentials, will reduce the frequency of occurrence of undetected abnormal cells.

Sysmex NE-8000 was much more sensitive for detecting immature granulocytes than Technicon H-1. In contrast, Technicon H-1 was more sensitive for detecting atypical lymphocytes. Measurement of left shift, traditionally expressed as the band cell count, has been a controversial subject for many years (15). The band cell count, which is severely limited by the difficulty of defining a band cell in precise terms, does not reliably reflect increased granulopoiesis. Keeping in mind the imprecision of band counts, their significance, particularly in the absence of other laboratory or clinical abnormalities, can be questioned. The left shift flag did show more numerical correlation with the band cell count by the Technicon H-1 than with the Sysmex NE-8000.

In this era of automated haematology analysers, the role of the manual leukocyte differential count may seem controversial. Manual differential counts are known to have a poor sensitivity and specificity (1, 16). Differences in cell classification criteria, nonrandom distributions of cell types in the smear, possibly inadequate sample size content and fatigue of the examiner are only a few of the factors influencing differential count imprecision (17, 18). However, in some cases cell abnormalities were missed by the Sysmex NE-8000, which were detected by manual examination. Errors were most prominent in detecting the presence of less than 2% atypical lymphocytes and in detecting left shift. The cost effectiveness of routine screening for such abnormalities and their clinical importance have not yet been assessed.

Sysmex NE-8000 can be made more sensitive for erythrocyte morphology and colour abnormalities, by adapting moving discriminators for red cell distribution width, mean corpuscular volume and mean corpuscular haemoglobin concentration.

During a two-week evaluation period, the Sysmex NE-8000 proved to be a very reliable instrument that was easy to handle and required minimal maintenance procedures. The computer of the NE-8000 is able to examine and evaluated the haematological data on each sample and provide interpretative comments. The Sysmex NE-8000 is a valuable aid in the clinical laboratory. In accurately distinguishing normal from abnormal specimens, it takes over the screening task from the haematology technician, leaving more time for the microscopic examination of those samples that deserve further attention.

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Dr. Katrien Devreese
Centraal Laboratorium
Universitair Ziekenhuis Gent
De Pintelaan 185
B-9000 Gent